SECRETOR STATUS AND FACTORS REGULATING URINARY TRACT ADHESION IN UROPATHOGENIC *ESCHERICHIA COLI* IN PATIENTS WITH DIABETES MELLITUS

A Synopsis of the thesis
Submitted for the award of
Degree of

DOCTOR OF PHILOSOPHY

In

MICROBIOLOGY

(Faculty of Medicine)

Submitted to

SRI BALAJI VIDYAPEETH

(Deemed -to-be- University)

PONDICHERRY- 607402

Submitted by

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December, 2020

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Introduction

Urinary Tract Infection (UTI) remains the most common bacterial infection encountered worldwide and also frequently occurring Nosocomial infection. UTI imposes a global economic burden on health care system with high morbidity.[1] UTI can classified in to two by anatomical site of infection, Upper and lower UTI. Upper UTI comprises Pyelonephritis presenting with chills, fever, flank pain along with systemic illness. Lower UTI comprises cystitis, prostatitis, urethritis presenting with dysuria, frequency and Urgency. UTI can also be classified as complicated and uncomplicated UTI depending upon the presentation.[2]

Uropathogenic Escherichia coli (UPEC)

UPEC always remain the most common etiological agent for UTI.[3] UPEC belongs the Enterobacteriaceae family with gram negative motile bacilli. UPEC originated in the gut migrates from anus to urinary tract and invades the urothelium with three types of adhesin factors namely type 1 fimbriae, P- fimbriae, X- adhesin and other factors like alpha hemolysin, cytotoxic necrotizing factor, serum resistance, endotoxin and biofilm formation.

Once UPEC adhere the uroepithelium it communicates with the adjacent bacterial colonies by Quorum sensing and form a sticky extra-cellular polysaccharide matrix called biofilm. This biofilm matrix makes the antimicrobial agents impermeable to host cell.[4]

Risk factors for UTI

UTI is more common in female due to various factors. Female have a shorter urethra than male which facilitates bacteria to travel a shorter distance to invade bladder and female urethral opening is in close proximity to anus. After menopause the decreased estrogen level makes the vaginal epithelium favorable for bacterial infection. Babies born with vesicourethral reflux causes urine backup in urethra which favors UTI.[5] Kidney stones, enlargement of

prostrate in men causes stagnation of urine and causes UTI. Urinary catheter use in case of bedridden patients permits bacteria to enter the bladder

Asymptomatic Bacteriuria

Urine culture with significant bacteriuria (>10^5cfu/ml) with pyuria is considered as UTI. But few patients have asymptomatic bacteriuria like pregnancy, children, people with Diabetes mellitus and elderly population. Treatment of asymptomatic bacteriuria remains a great challenge.[3]

Antibiotic resistance

Increasing antibiotic resistance is due to selection pressure is related to inadequate empirical therapy without prescription finally end up in ineffective UTI treatment.[6] High level of antibiotic resistance to UPEC is reported to Cotrimoxazole and Ciprofloxacin with emerging resistance to third generation cephalosporins worldwide.[7]

Secretor status and its disease association

Persons with secretion of blood group antigen in body fluids are called secretors. Absence of respective antigen are called non-secretors. Secretor refers to H antigens which is indirect gene products expressed as fucose-containing glycan units resides on glycoproteins or glycolipids of erythrocyte membranes or on mucin glycoproteins in secretions. H antigen expressed by 2 genes FUT1(H) gene predominantly in erythroid tissues; FUT2 (Secretor) gene is expressed predominantly in secretory tissues.[8]

Non-secretor has increased inflammatory response (increased C-reactive protein, erythrocyte sedimentation rate and the body temperature) to UTI than Secretors.[9] Non-secretors synthesize unique glycolipid receptor on their vaginal epithelial cells causes increased adherence to *E. coli* and increased risk for recurrent UTI. Secretor status predisposing to several

diseases involving mucus layers such as inflammatory bowel diseases as well as other multi-system diseases such as Rheumatic fever and rheumatic heart disease.[10]

1. Aims and Objectives

Primary Objectives

1. To identify ABO secretor status in patients with significant bacteriuria among diabetics and non-diabetics

2. To identify the relationship between ABO secretor status and diabetes mellitus with the phenotypic and genotypic characteristics of virulence factors regulating Uropathogenic *Escherichia coli* in Urinary tract infections

3. To study the relationship between ABO secretor status and diabetes mellitus with phenotypic and genotypic features of antibiotic resistance in Uropathogenic *Escherichia coli* (UPEC)

Secondary Objectives

4. To study the association of ABO secretor status, diabetes mellitus antibiotic resistance and virulence factors of UPEC with renal dysfunction at diagnosis and end of follow up, need for and duration of hospital admission

5. To assess the ability of Neutrophil lymphocyte ratio of blood and Urine microscopy score in independently predicting renal dysfunction at presentation and at end of follow up, need for and duration of hospital admission

2. Review of Literature

Urinary tract is protected from infections by several host factors such as regular voiding, protective factors in the urothelial lining such as mucus secretions, bactericidal activity of urinary constituents such as urea, immunoglobulins in mucosal surface such as IgA and several other factors. The most important factor that predisposes urinary tract infection is increased adherence and one of the factors that has been implicated in this regard is the secretion of blood group antigens in various body fluids. ABO blood group secretor status in body fluids is tested by adsorption-inhibition method, and it is possible to semi-quantitatively assess the presence of secretor status. Non-secretors who lack the presence of blood group antigens in secretions are predisposed to increased adherence of bacteria, though the exact mechanism is not fully understood. It is hypothesized that presence of blood group antigens causes significant stearic interference in the adherence of uropathogens. The blood group secretor status and their levels significantly vary with time and hence it is difficult to study and understand the effect of this secretor status in control individuals and it needs to be studied during disease condition.[5]

In view of the considerable phenotypic variations in the secretor status with time, secretor status has been studied by assessing the FUT2 and FUT3 genes either through single nucleotide polymorphisms or through genome wide association studies. There has been several reports on the role of genetic variations in these two genes with influences in innate and adaptive immunity, predisposing to several diseases involving mucus layers such as inflammatory bowel diseases as well as other multi-system diseases such as Rheumatic fever and rheumatic heart disease.[10,16,17,18]

Diabetes mellitus is a common non-communicable disease in India and world and is a major public health problem with multi-system organ and visceral damage with damage to both

micro-vasculature and medium to large sized blood vessels and nerves. Diabetes predisposes a patient to UTI by several mechanisms including presence of glucose in urine and dysfunctional motility of the bladder due to neuropathy in addition to several other factors promoting bacterial growth and adhesion in addition to damage to protective factors such as mucosal as well as systemic immunity in the body. In addition, there is higher colonization of pathogenic bacteria in the gut, perineum and in vaginal flora, thereby increasing the chance of both primary infection, reinfection and predisposing to ascending infections resulting in pyelonephritis and kidney damage.[5,19,20]

Prevalence of pyelonephritis is higher in diabetics (9.4 %) compared with non-diabetic patients (3.2 %).[11] Diabetics with UTI also presents with asymptomatic bacteriuria (ASB) (2 cultures at least 24 hours apart, yielding $\geq 10^5$ colony forming units/milliliter (CFU/ml) with same organism. ASB can predispose to recurrent UTIs, progressive renal dysfunction, hypertension and higher mortality.

Urinary tract infection (UTI) is defined as the presence of $\geq 10^5$ colonies of one bacterial pathogen per ml of a clean midstream urine (MSU) with or without symptoms and signs such as loin pain, tenderness, fever, frequency, urgency and dysuria. Diagnosis of acute pyelonephritis and cystitis was made according to the presence or absence of fever and loin pain or tenderness respectively.[2,21]

Older age and female sex have also been identified as major predisposing factors for UTI due to increased comorbidities such as diabetes in old age and anatomical and hormonal factors in female patients such as shorter length of urethra, hormonal changes, sexual practices and child birth in reproductive age group, lack of breast feeding etc. and several other factors. [5,20]

Bacterial virulence and antibiotic resistance are the major bacterial factors determining the pathogenicity, severity of disease, predisposition to upper urinary tract infection causing pyelonephritis and renal dysfunction, need for and duration of hospitalization. It is well known that co-morbidities such as diabetes mellitus increases the chance of resistance to antibiotics such as Ofloxacin and cefixime.[22] Higher percentage of resistance is also reported with common anti-microbials such as amoxicillin, nitrofurantoin, trimethoprim/sulfamethoxazole and ciprofloxacin in diabetic patients with ASB.[23] Gestational diabetes mellitus, however was not associated either with risk of UTI nor maternal and perinatal morbidity.[21]

In another study comparing the health care costs of UTI in diabetes mellitus patients from US, DM was associated with higher medical expenditure in UTI patients.[1] Surface virulence factors regulating type I fimbriae binding to urothelial uroplakin Ia and IIIa (UPIIIa) via adhesin subunit FimH encoded by FimH gene, P fimbriae, regulating pathogenesis of ascending UTIs and pyelonrphritis caused by UPEC in humans encoded by pap-A-K gene operon, are important factors determining the extent of renal damage and morbidity due to UTI. Among the secreted virulence factors, E.coli mediated lipoprotein alpha-hemolysin, encoded by hlyA and cytotoxic necrotising factor (CNF1) are commonly associated with upper UTI and pyelonephritis.[24]

Type 2 DM patients with ESBL-E.coli had higher peripheral blood neutrophil lymphocyte ratio and these patients were associated with higher all causes mortality than those with K. pneumoniae UTI.[25]

Urine microscopy is a non-invasive tool for the diagnosis and management of several conditions affecting the kidneys and urinary tract. Perazella [12] proposed a scoring system which predicts acute kidney injury and also gives prognostic value according to the score.[12]

Similar predictive ability of Urine microscopy scoring system was found in sepsis induced AKI in several other studies.[26]

3. Materials and Methods

Study Population – Humans

Type of study – Prospective Analytical Study

Place of Study – Indira Gandhi Govt. General Hospital & Postgraduate Institute, Mahatma Gandhi Medical College & Research Institute and Vellore Institute of Technology, Vellore

Study population

Inclusion Criteria –

- Patients > 18 years old
- Patients with Urinary tract infections with and without diabetes mellitus
- Patients who give informed consent for the study
- Follow up available for at least 14 days from the date of diagnosis

Exclusion Criteria:

- Antenatal women
- Patients with inadequate clinical details
- Patients in whom both blood specimen and urine culture samples are not available

Study Group

- Patients with significant bacteriuria (>105 CFU/ml) with pyuria (> 10 WBC's/HPF)
- Diabetic and Non diabetic
- Secretor and Non-Secretor

Sample size Calculation:

- Significant Bacteriuria - Assuming 5% alpha error (95% significance level) with a power of 90%, expected proportion of UTI being non-secretor as 41.5% and proportion of control as 22.6%, we need **137** individuals in each group.
- Sample size for E coli positivity in Diabetics and Non-diabetics: Assuming alpha error of 5%, expected proportion of culture positive UTI patients with *E.coli*,
- 25%, absolute precision of 5%, we need **289** culture positive UTI patients for the study
- Considering sub-group analysis and adjusted analysis, we intend to recruit 289 patients (**rounding of to 300).**

Approvals obtained

- Study was approved by Institute's Scientific Advisory Committee
- Institutional Ethical Clearance obtained (No. GHIEC/2016 dated 22/12/2016)

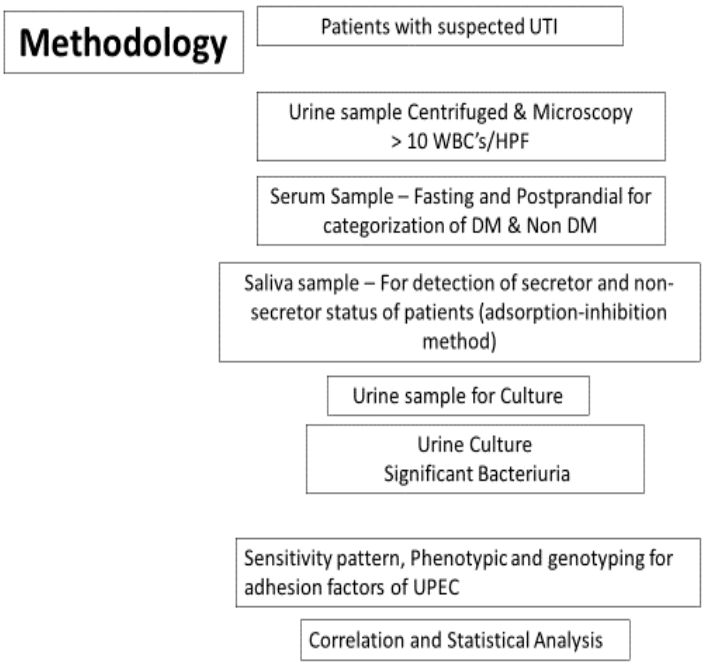
Research Methodology course

- Basic modules and module on how to write a research paper completed
- Five modules completed from online course of British Medical Journal and University of California
- Reference website www.rtop.bmj.com

Outline:

After obtaining informed consent

- Urine samples from patients with suspected UTI has been centrifuged and examined for pyuria (>10 WBC's/HPF). Urine samples with pyuria were cultured on Agar media and samples with significant bacteriuria with E. coli as isolate were included in the study and informed consent obtained
- The blood group of the patients were assessed by test tube method using 5% of red cell suspension with antiserum A, B, AB and Rhesus antibody
- Plasma has been collected for assessment of complete blood count to obtain Neutrophil-lymphocyte ratio
- Secretor status of the patients was determined from saliva by adsorption inhibition method (Suha T. Abd., 2015)
- Serum was collected for assessment of blood sugar in fasting and post prandial levels and Urea and creatinine levels
- Subjects followed over a period of 14 days and monitored for systemic illness
- E.coli isolates will be processed for phenotypic and genotypic study of adhesion factors and Antibiotic resistance
- Healthy donors from blood bank were screened for secretor status from saliva and blood group was assessed after taking informed consent


Methodology
Patients with suspected UTI
Urine sample Centrifuged & Microscopy
> 10 WBC's/HPF
Serum Sample – Fasting and Postprandial for categorization of DM & Non DM
Saliva sample – For detection of secretor and non-secretor status of patients (adsorption-inhibition method)
Urine sample for Culture
Urine Culture
Significant Bacteriuria
Sensitivity pattern, Phenotypic and genotyping for adhesion factors of UPEC
Correlation and Statistical Analysis

Clean catch mid-stream urine is collected in a sterile wide mouthed container of capacity 30ml.Urine samples are subjected to microscopy and inoculated in MacConkey agar and Blood agar using a sterile 4 mm platinum wired calibrated loop and incubated overnight at 37ºC for 24 hours. The specimen with significant bacteriuria (> 10^5colony forming unit per ml) and pyuria (>10 WBC's/HPF) were considered as UTI and processed for identification of organism by standard biochemical test. Antibiotic sensitivity testing was done following the Kirby-Bauer disc diffusion method in Muller Hinton agar as per Clinical and Laboratory Standards Institute (CLSI) guidelines.[11] Cultures with UPEC were isolated and stored in LB broth till further analysis

Scoring by Urine Microscopy

Ten ml of urine was centrifuged at 1500rpm for 5min, urine sediment was applied to a glass slide and coverslip was gently applied. The slide was observed in light microscope under high power field (40X) and analysed for red and white blood cells, renal tubular epithelial cells (RTEC), granular cast and hyaline casts. RTEC are defined as size variation (11 to 15 _m in diameter), shape variation (round to columnar), and a well-evident nucleus with nucleoli.

Granular casts are defined as fine or coarse granules contained within a cast matrix, whereas hyaline casts are defined as cast matrix without cells and colourless. Granular casts and RTEC per high-power field were recorded on the data collection sheet as present or absent and, when present,

were also quantified as the number counted: one to five, six to 10, and >10 and one to five, six to 20, >20, respectively.[12]

Table 1: Perazella scoring system based on number of granular cast and RTEC seen under high-power field

Score	**description**
1	RTE cells 0 and granular casts 0
2	RTE cells 0 and granular casts 1to 5 or RTE cells 1 to 5 and granular casts 0
3	RTE cells 1 to 5 and granular casts 1 to 5 or RTE cells 0 and granular casts 6 to 10 or RTE cells 6 to 20 and granular casts 0

Note: Score of ≥2 represents strong predictor of Acute Kidney Injury

Phenotypic characterisation of Virulence factors

UPEC has various virulence factors to resist host immune response. Most commonly used virulence factors were selected and included in the study.

Hemolysin Production

The E. coli isolates were inoculated on 5% sheep blood agar and incubated over night at 37^{o}C. The indicator of hemolysin production was the presence of a zone of complete lysis of erythrocytes around the colony and clearing of the medium.

Hemagglutination

The test was carried out as per the direct bacterial hemagglutination test-slide method. One drop of RBC suspension was added to a drop of broth culture and the slide was rocked at room temperature for 5 minutes. Presence of clumping was taken as positive for hemagglutination. Mannose-sensitive hemagglutination was detected by the absence of hemagglutination in a parallel set of tests in which a drop of 2% W/V D-mannose was added to the red cells and a drop of broth culture. MRHA was detected by the presence of hemagglutination of 3% 'O' blood group human RBCs in the presence of 2% W/V D-mannose.

Serum resistance

Overnight growth of E. coli on 5% sheep blood agar was suspended in PBS to produce a count of 2.5x104 cfu/ml. 10µl of the suspension was then inoculated on 5% sheep blood agar plates. In a test tube 50µl of bacterial suspension is mixed with 50µl of human serum and is incubated for a period of 180 minutes. After incubation 10µl of this suspension was inoculated on 5% blood agar and incubated overnight at 37°C and the viable count was determined. Susceptibility of bacteria to serum bactericidal activity was expressed as the percentage of bacteria surviving after 180 minutes in relation to the original count of bacteria determined at 0 minutes. Strains were considered as serum sensitive if the viable count dropped by 1% of the initial value and resistant if > 90% of organisms survived after 180 minutes.

Biofilm production

Biofilm production assay on Congo red agar

The isolates were cultured on CRA plates, prepared by adding 0.8 g of Congo red stain and 36 g of sucrose to 1 L of BHI agar. After 24 h incubation at 37°C, plates were further incubated for 12 h at room temperature. isolates with black colonies were considered to be biofilm-producers.

Crystal violet method

Isolates from fresh agar plates were inoculated in LB broth and incubated for 24 h at 37°C, then diluted with fresh LB broth in 1 in 100 dilution. Individual wells of sterile, polystyrene, 96 well-flat bottom tissue culture plate (TCP) wells filled with 0.2 ml aliquots of the diluted cultures and only broth served as control to check sterility and nonspecific binding of media. The TCP was incubated for 18–24 h at 37°C. After incubation content of each well was gently removed by tapping the plates. Then wells were washed four times with 0.2 ml of PBS (pH 7.2) to remove free-floating "planktonic" bacteria. Wells were stained with crystal violet (0.1%). Excess stain was rinsed off by washing with sterile distilled water, and the plate was kept for drying. If biofilm is formed by organisms, then wells are uniformly stained with crystal violet. Optical density (OD) of stained adherent bacteria was determined with ELISA reader at a wavelength of 570 nm. Experiment was repeated thrice, and the data then were averaged. The mean OD value obtained from media control was deducted from all the test OD values.

ESBL Production

E. coli isolates are subjected to combined disk method with Cefotaxime (30µg), Cefotaxime-clavulanate(30/10µg) and ceftazidime (30µg), ceftazidime-clavulanate (30/10µg) as per CLSI guidelines.[10]

Genotypic characterisation of Virulence factors

DNA Extraction

bacteria grown overnight inoculated in a test tube containing one ml of distilled water and boiled for 10 minutes in a water bath at 100^{o}C, and then were centrifuged for five minutes

at 1000 rpm. Clear supernatant which is the DNA extract was collected in cryovials as aliquots and stored at -80^{o}C for further analysis.[13]

Real Time PCR

Ten genes were recruited for analysis of antibiotic resistance and virulence factors and performed Real Time PCR. Genes studied for β-lactamases (blaSHV, blaCTX-M, metallo β-lactamases (NDM1), type 1 fimbriae (fimH), P fimbriae (papA), serum resistance (iss), hemolysin (hlyA), cytotoxic necrotising factor (cnf1), curli fimbriae (csgA) and biofilm (iutA). The *TaqMan* probes was used and Real Time PCR performed with Applied Biosystem Quant Studio 3 instrument. All the primers and probes were selected from the National Center for Biotechnology Information website (NCBI; http://www.ncbi.nlm.nih.gov) and synthesized by Helini Biomolecules. The primers were checked for specificity in a BLAST search available through the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Experiments are repeated thrice to check accuracy

Table: 2 Primer and Probe sequence of genes for Real Time PCR

Sl.no	Gene	Primer		bp size
1	blaSHV	Forward	CGGTCAGCGAAAAACACC	139bp
		Reverse	CGCAAAAAGGCAGTCAATC	
		probe	CCGCCATTACCATGAGCGATAACAG	
2	blaCTX-M	Forward	GACGTTAAACACCGCCATTC	148bp
		Reverse	GGTGGTATTGCCTTTCATCC	
		probe	CACTTCACCTCGGGCAATGGCG	
3	NDM1	Forward	GTCTGGCAGCACACTTCCTA	99bp
		Reverse	CGCCATCCCTGACGATCAAAC	
		probe	TCTCGACATGCCGGGTTTCGG	
4	fimH	Forward	TTTTGCGACAGACCAACAAC	119bp
		Reverse	GGTGACATCACGAGCAGAAA	
		probe	AATAATGATGTGGTGGTGCCCACTG	
5	papA	Forward	CAGATATCTCTGGTGTGTTCAGT	122bp
		Reverse	GGAATAGTTGGAGCAGCATTTAC	
		probe	TTATTGCCGGTGCGGTAGCTATGG	
6	iss	Forward	CCGACAGCAGTAACACCAAA	156bp
		Reverse	ACCGAGCAATCCATTTACGA	

		probe	CACTCATCATTTCTTCGTTTCGGGAA	
7	hlyA	Forward	AGAAACTCATTGGCCTCACC	147bp
		Reverse	GTACACTGCCTGCCTTTCCT	
		probe	AAAGCGGGTAATAAATTAGGCGGCA	
8	cnf1	Forward	TTCTCTGGACTCGAGGTGGT	175bp
		Reverse	TGCGGTAATTTTGGGTTTGT	
		probe	CCTATTCTGCAGTGACCGGATCTCC	
9	csgA	Forward	GCAGCAATCGTATTCTCTGGTAG	85bp
		Reverse	TATTACCGCCACCACCGT	
		probe	TGGCAGGTGTTGTTCCTCAGTACG	
10	iutA	Forward	ATTCAGGGCGGCAAAGAG	160bp
		Reverse	GGCTGTCGGTACGTGAAGAG	
		probe	ACTACGGTATGAATGTGCGTGGCC	

Blood investigations

Blood samples were tested for fasting, post-prandial sugar levels, urea, creatinine, complete blood count using fully automated analyser (Beckman Coulter) and neutrophil-lymphocyte ratio calculated

Neutrophil-Lymphocyte ratio (NLR)

$$NLR = \frac{\text{Absolute neutrophil count}}{\text{Absolute lymphocyte count}}$$

NLR $\geq$ 4.2 was a predictor of Acute kidney injury.[14]

Identification of Secretor status

Blood was collected in ethylene diamine tetra acetic acid (EDTA) coated vacutainer tubes are processed for ABO blood group and Rh typing by forward and reverse grouping techniques using test tube method**.** Secretor status of the saliva samples were identified by using adsorption inhibition method. The procedure is briefly mentioned below.

Six test tubes were labelled C, 2, 4, 8, 16, 32. C refers to the control tube, to which no saliva was added. 50 µl of saliva was added into the second tube labelled 2. For the rest of the tubes labelled 4, 8, 16 and 32, saliva was titrated by doubling dilution using normal saline. 50 µl of anti A serum was added to each test tube. All the tubes were shaken well and left

undisturbed for 10-15 minutes. One drop of a suspension of group A RBCs in a concentration of 5% was added to each tube. The tubes were left to stand for 5 minutes and subsequently centrifuged at 3500 rpm for 20 seconds. The sediment was decanted on a slide and studied for presence of agglutination with microscopy. The degree of agglutination was graded as follows,

Grade 0 - all red blood cells are discrete and evenly distributed in surrounding fluid (no agglutination)

Grade 1 - Few or very occasional clumps

Grade 2 - moderate aggregates

Grade 3 - composed of moderate to large aggregates

Grade 4- very large aggregates, very few cells remain free without agglutination.

Control tubes always showed grade 4 Agglutination. The procedure was repeated with group B RBC and anti B serum and subsequently with group O RBCs and anti H serum. This is based on the principle that when saliva has blood group antigens, the antibodies in the serum of appropriate dilution will be utilized and will result in a negative agglutination when the corresponding blood group RBC's are added. Grades 0, 1, 2, 3 agglutination are interpreted as secretor while Grade 4 agglutination is interpreted as non-secretor.[15]

Statistical Analysis:

Done by STATA software

4. Results:

Among 300 UTI patients due to UPEC 128 were male and 172 were females. 53 cases came as outpatients where 247 cases were hospitalized

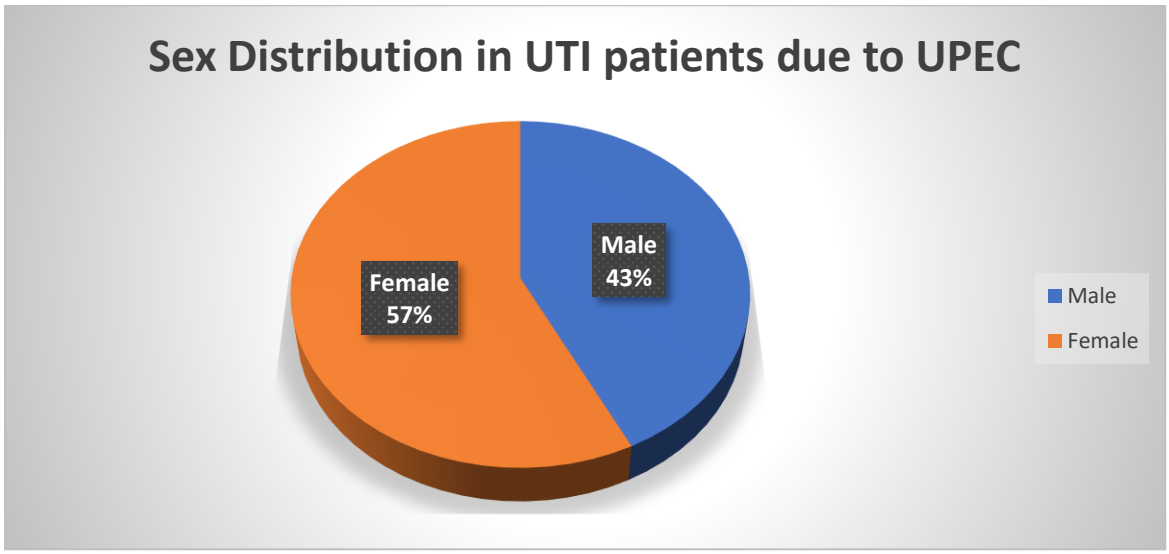
Epidemiological Distribution

Sex

Male 128 (42.7%)

Female 172 (57.3%)

Figure:1 Sex distribution in UTI

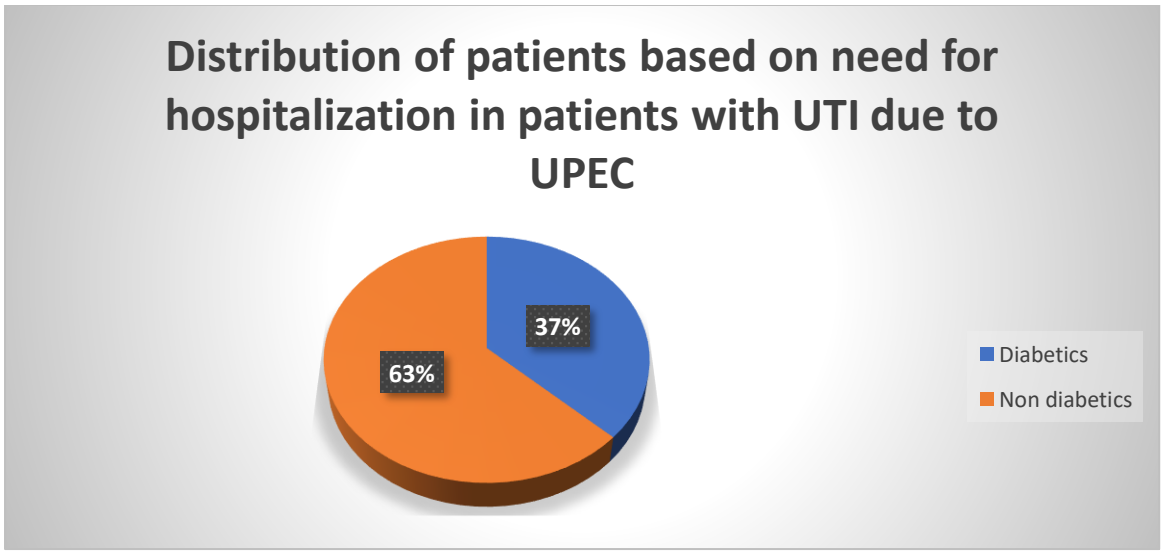


Hospital stay

Inpatients 247 (82.3%)

Outpatients 53 (17.7%)

Figure:2 Distribution of UTI patients based on need for hospitalization


Distribution of patients based on need for hospitalization in patients with UTI due to UPEC
37%
63%
Diabetics
Non diabetics

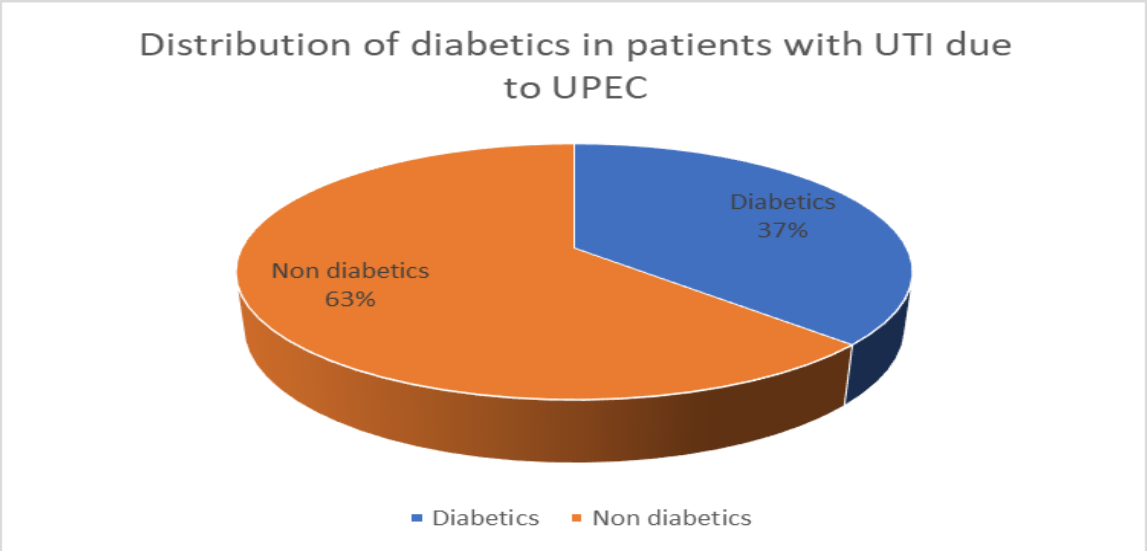
Out of 300 patients with UTI due to UPEC 111 cases were diabetics and 189 were non-diabetics. Patients with renal dysfunction at presentation were 99 out of 300 patients

Diabetes Mellitus

Present 111 (37%)

Absent 189 (63%)

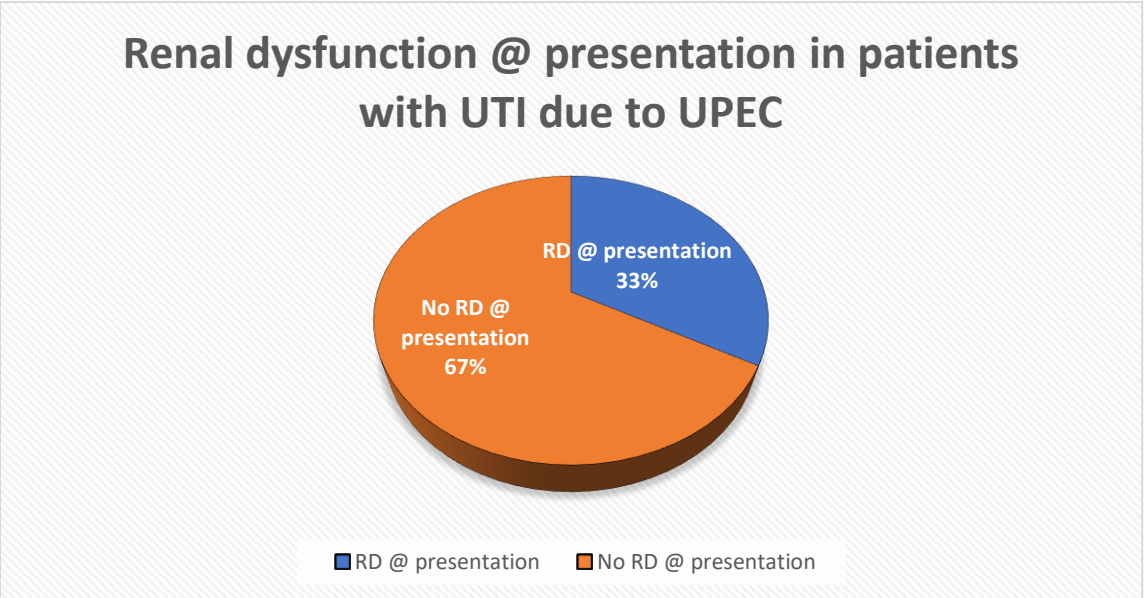
Figure:3 Distribution of diabetics in patients with UTI



Renal dysfunction at presentation

Present 99 (33%); Absent 201 (67%)

Figure: 4 Distribution of Renal dysfunction at presentation in patients with UTI


Renal dysfunction @ presentation in patients with UTI due to UPEC
RD @ presentation 33%
No RD @ presentation 67%
RD @ presentation
No RD @ presentation

Objective 1 - RESULTS

1. To identify ABO secretor status in patients with significant bacteriuria among diabetics and non-diabetics

5.1 Secretor and Diabetics status in UTI by UPEC

In our study with UTI by UPEC Non-secretors were more common in O blood group followed by B, A and AB (Table 2). Non-secretor has influence in diabetics and renal dysfunction which is statistically significant (Table 3)

Table 3: Correlation of ABO Blood group and Secretor status in Saliva by Adsorption-Inhibition Method

Blood group	**Non-secretor N (%)**	**Secretor N (%)**	Pearson Chi^2 P value
O	99(79.8%)	25(20.2%)	0.000
A	21(40.4%	31(50.6%)	
B	54(55.7%)	43(44.3%)	
AB	9(33.3%)	18(66.7%)	

Note: Non-secretors are more common in O group followed by B, A and AB

Table 4: Correlation of ABO blood group secretor status with sex, diabetic status and renal dysfunction in patients with UTI

Clinical Parameters	**Non-secretor N (%)**	**Secretor N (%)**	Pearson Chi^2 P value
Sex			
Male	83(64.8%)	45(35.2%)	0.239
female	100(58.1%)	117(41.9%)	
Diabetes mellitus			
Non-DM	97(51.3%)	92(48.7%)	0.000
DM	86(77.5%)	25(22.5%)	
Renal dysfunction			0.000
Absent	104(51.7%)	97(48.3%)	
present	79(79.8%)	20(20.2%)	

Objective 2 - RESULTS

2. To identify the relationship between ABO secretor status and diabetes mellitus with the phenotypic and genotypic characteristics of virulence factors regulating Uropathogenic *Escherichia coli* in Urinary tract infections

5.2 Virulence factors in UTI by UPEC

Virulence studies shows higher expression of serum resistance followed by biofilm production, MRHA, hemolysin and MSHA (Table 4)and phenotypic studies correlates with prevalence of genotyping (Table 5)

Table 5: Expression of Virulence factors in UPEC

Virulence Factors	Absent	present
Hemolysin	222(74%)	78(26%)
MSHA	237(79%)	63(21%)
MRHA	167(55.7%)	133(44.3%)
Serum resistance	58(19.3%)	242(80.7%)
Biofilm production	104(34.7%)	196(65.3%)

Note: MSHA- Mannose sensitive hemagglutination

MRHA- Mannose resistant hemagglutination

Table 6: Prevalence of Virulence genes in UPEC

Virulence genes	Absent	Present
hlyA	216(72%)	84(28%)
cnf1	181(60.3%)	119(39.7%)
iss	234(78%)	66(22%)
fimH	238(79.3%)	63(20.7%)
papA	174(58%)	126(42%)
iutA	170(56.7%)	130(43.3%)
csgA	81(27%)	219(73%)

5.3 Association of Virulence factors with Secretor and Diabetic status

All the virulence factors studied by both phenotypic findings and the presence of corresponding genes, were significantly influenced in non-secretors (Table 6 &7)

Table 7: Correlation of Secretor status with phenotyping of virulence genes of E coli in UTI patients

Secretor Status	**Hemolysin**		Pearson Chi^2 p-value		**Hemolysin**		Pearson Chi^2 p-value
	Absent	present			Absent	present	
Non-Secretor	116(63.4%)	67(36.6%)	0.000	Non-DM	141(74.6%)	48(25.4%)	0.756
Secretor	106(90.6%)	11(9.4%)		DM	81(73%)	30(27%)	
Total	222	78		Total			
	MSHA				**MSHA**		
Non-Secretor	135(73.8%)	48(26.2%)	0.005	Non-DM	150(79.4%)	39(20.6%)	0.839
Secretor	102(87.2%)	15(12.8%)		DM	87(78.4%)	24(21.6%)	
Total	237	63		Total	79	21	
	MRHA				**MRHA**		
Non-Secretor	74(40.4%)	109(59.6%)	0.000	Non-DM	112(59.3%)	77(40.7%)	0.102
Secretor	93(79.5%)	24(20.5%)		DM	55(49.5%)	56(50.5%)	
Total	167	133		Total	167	133	
	Serum resistance				**Serum resistance**		
Non-Secretor	34(18.6%)	149(81.4%)	0.899	Non-DM	43(22.7%)	146(77.3%)	0.107
Secretor	24(20.5%)	93(79.5%)		DM	15(13.5%)	96(86.5%)	
Total	58	242		Total	58	242	
	Biofilm production				**Biofilm production**		0.260
Non-Secretor	46(25.1%)	137(74.9%)	0.000	Non-DM	70(37%)	119(63%)	
Secretor	58(49.6%)	59(50.4%)		DM	34(30.6%)	77(69.4%)	
Total	104	196		Total	104	196	

Table 8: Correlation of Secretor status with genotyping of virulence genes of E coli in UTI patients

Secretor Status	***hlyA* gene**		Pearson Chi^2 P value
	Absent N (%)	Present N (%)	
Non-Secretor	108 (59%)	75(41%)	Pearson Chi2 = 39.23 P = 0.000
Secretor	108 (92.3%)	9(7.7%)	
Total	216	84	
	***cnf1* gene**		
Non- Secretor	93 (50.8%)	90 (49.2%)	Pearson Chi2 = 17.74 P = 0.000
Secretor	88 (75.2%)	29 (24.8%)	
Total	181 (60.3%)	119(39.7%)	
	***iss* gene**		
Non- Secretor	134 (73.2%)	49 (26.8%)	Pearson Chi2 = 6.23 P = 0.013
Secretor	100 (85.5%)	17(14.5%)	
Total	234 (78%)	66(22%)	
	***fimH* gene**		
Non-Secretor	135 (73.8%)	48(26.2%)	Pearson Chi2 = 8.8 P = 0.003
Secretor	103 (88%)	14(12%)	
Total	238(79.3%)	62(20.7%)	
	***papA* gene**		
Non- Secretor	76 (41.5%)	107 (58.5%)	Pearson Chi2 =52.25 P = 0.000
Secretor	98 (83.8%)	19 (16.2%)	
Total	174 (58%)	126(42%)	
	***iutA* gene**		
Non- Secretor	87 (47.5%)	94 (51.3%)	Pearson Chi2 = 16.49 P = 0.0698
Secretor	83 (71%)	34(29%)	
Total	170 (56.67%)	128(42.67%)	
	***csgA* gene**		
Non- Secretor	44 (24%)	139 (76%)	Pearson Chi2 = 2.08 P = 0.149
Secretor	37 (31.6%)	80(68.4%)	
Total	81 (27%)	219(73%)	

Objective 3 - RESULTS:

1. To study the relationship between ABO secretor status and diabetes mellitus with phenotypic and genotypic features of antibiotic resistance in Uropathogenic *Escherichia coli* (UPEC)

5.4 Antibiotic resistance in UTI by UPEC

Higher level resistance was seen in fluroquinolone, cotrimoxazole, and β-lactam group of drugs whereas, low level resistance was seen in nitrofurantoin, amikacin and Meropenem. Fosfomycin shows 100% sensitivity. These results were corresponding with genotyping (Table 8). 53% of isolates were ESBL producer.

Table 9: Antibiotic resistance pattern in UTI by UPEC

	Sensitive	Resistant
Ampicillin	91(30.3%)	209(69.7%)
Amikacin	249(83%)	51(17%)
Ceftriaxone	94(31.3%)	206(68.7%)
Cotrimoxazole	96(32%)	204(68%)
Fosfomycin	300(100%)	0(0%)
Nitrofurantoin	260(86.7%)	40(13.3%)
Norfloxacin	34(11.3%)	266(88.7%)
Meropenem	240(80%)	60(20%)

5.5 Association of antibiotic resistance with diabetic and secretor status

β-lactam group of drugs and its corresponding genes and ESBL production shows statistically significant association with Non-secretors whereas, diabetic status won't have association with any of the antibiotics.

Table 10: Association of Resistance to antibiotics in secretor and diabetic status

Antibiotic resistance	Secretor status		Pearson Chi^2 P value	Diabetic status		Pearson Chi^2 P value
	Non-secretor	secretor		Non-DM	DM	
Ampicillin	147(80.3%)	62(52.9%)	0.000	129(68.2%)	80(72.1%)	0.487
Amikacin	29(15.8%)	22(18.8%)	0.506	38(20.1%)	13(11.7%)	0.062
Ceftriaxone	145(79.2%)	61(52.1%)	0.000	128(67.7%)	78(70.3%)	0.646
Cotrimoxazole	130(71%)	74(63.2%)	0.158	127(67.2%)	77(69.4%)	0.697
Fosfomycin	0	0	-	0	0	-
Nitrofurantoin	23(12.6%)	17(14.5%)	0.626	30(15.9%)	10(9%)	0.091
Norfloxacin	159(86.9%)	107(91.4%)	0.223	168(88.9%)	98(88.3%)	0.874

Meropenem	40(21.9%)	20(17.1%)	0.314	35(18.5%)	25(22.5%)	0.403
ESBL production	115(62.8%)	45(38.5%)	0.000	98(51.8%)	62(55.9%)	0.706

Table 11: Presence of Antibiotic resistance genes in secretor and diabetic status

Gene	**Secretor status**		Pearson Chi^2 P value
blaSHV	**Non-secretor**	**secretor**	
Absent	155(84.7%)	90(76.9%)	
Present	28(15.3%)	27(23.1%)	0.090
blaCTX-M			
Absent	53(29%)	83(71%)	0.000
Present	130(71%)	34(29%)	
NDM1			
Absent	167(91.3%)	115(98.3%)	0.012
Present	16(8.74%)	2(1.71%)	

Secondary Objective

Objective 4 – RESULTS

To study the association of age, sex, blood group, ABO secretor status, diabetes mellitus, antibiotic resistance and virulence factors of UPEC with renal dysfunction at presentation and end of follow up, need for and duration of hospital admission

5.6 Distribution of Epidemiological factors in Renal dysfunction at presentation and at end of follow up

Older age, O blood group and male patients have more predisposition to renal dysfunction

Table 12: Association of Age of the patient with Renal dysfunction at presentation and at 14 days follow up

Age category in years	Renal dysfunction at presentation Pearson Chi2		Pearson Chi^2 P value	Renal dysfunction at 14 days follow-up Pearson Chi2		Pearson Chi^2 P value
	Absent	Present		Absent	Present	
13-25	22(100%)	0		22(100%)	0	
26-65	137(70.6%)	57(29.4%)	0.000	159(82%)	35(18%)	0.012
66-85	39(50%)	39(50%)		55(70.5%)	23(29.5%)	
>85	3(50%)	3(50%)		4(66.7%)	2(33.3%)	
Total	201(67%)	99(33%)		240(80%)	60(20%)	

Table 13: Association of Sex of the Patient with Renal dysfunction at presentation and at 14 days follow up

Sex	Renal dysfunction at presentation		Pearson Chi2 P value	Renal dysfunction at 14 days follow up		Pearson Chi^2 P value
	Absent	Present		Absent	Present	
Male	76(59.4%)	52(40.6%)	0.015	99(77.3%)	29(22.7%)	0.321
Female	125(72.7%)	47(27.3%)		141(82%)	31(18%)	
Total	201	99		240	60	

Table 14: Association of ABO Blood group of patients with renal dysfunction at presentation and at 14 days follow up

ABO Blood group	Renal dysfunction at presentation		Pearson Chi2 P value	Renal dysfunction at 14 days follow-up		Pearson Chi^2 P value
	Absent	Present		Absent	Present	
O	66(53.2%)	58(46.8%)	0.000	90(72.6%)	34(27.4%)	0.018
A	40(77%)	12(23%)		41(78.9%)	11(21.1%)	
B	76(78.3%)	21(21.7%)		87(89.7%)	10(10.3%)	
AB	19(70.4%)	8(29.6%)		22(81.5%)	5(18.5%)	
Total	201(67%)	99(33%)		240(80%)	60(20%)	

5.7 Association of diabetic status in Renal dysfunction at presentation and at end of follow-up

In patients with UTI by UPEC, diabetics has more predisposition to renal dysfunction at presentation and at end of follow up which was statistically significant

Table 15: Association of Diabetic and Secretor status of the patient with renal dysfunction at presentation and at 14 days follow up

Diabetes mellitus	Renal dysfunction at presentation		Pearson Chi2 P value	Renal dysfunction at 14 days follow up		Pearson Chi^2 P value
	Absent	Present		Absent	Present	
Absent	140(73%)	49(26%)	0.001	162(85.7%)	27(14.3%)	0.001
Present	61(55%)	50(45%)		78(70.3%)	33(29.7%)	
Total	201(67%)	99(33%)		240(80%)	60(20%)	

5.8 Association of virulence and antibiotic resistant gene of UPEC with renal dysfunction at presentation and at end of follow up

UPEC virulence and antibiotic resistant genes does not have direct association on renal dysfunction except iutA gene encoding biofilm production which has implication to end up with persistent renal dysfunction

Table 16: Association of virulence and antibiotic resistant gene of UPEC with renal dysfunction at presentation and at 14 days follow up

Gene	Renal dysfunction at presentation		Pearson Chi2 P value	Renal dysfunction at 14 days follow-up		Pearson Chi^2 P value
	Absent	Present		Absent	Present	
hlyA	55(65.5%)	29(34.5%)	0.726	67(79.7%)	17(20.2%)	0.949
cnf1	83(69.7%)	36(30.3%)	0.412	95(79.8%)	24(20.2%)	0.953
iss	39(59.1%)	27(40.9%)	0.122	49(74.2%)	17(25.8%)	0.185
fimH	41(66.1%)	21(33.9%)	0.870	50(80.6%)	12(19.4%)	0.887
papA	83(65.9%)	43(34.1%)	0.724	97(77%)	29(23%)	0.266
iutA	81(62.3%)	49(37.7%)	0.131	93(71.5%)	37(28.5%)	0.001
csgA	146(66.7%)	73(33.3%)	0.840	180(82.2%)	39(17.8%)	0.119
blaSHV	39(70.9%)	16(29.1%)	0.495	43(78.2%)	12(21.8%)	0.709
blaCTX-M	107(65.2%)	57(34.8%)	0.477	134(81.7%)	30(18.3%)	0.417
NDM1	13(72.2%)	5(27.8%)	0.627	15(83.3%)	3(16.7%)	0.715

5.9 Distribution of epidemiological factors in duration of hospital stay

Hospital stay was categorized as need for admission, stay less than 5 days and stay more than 5 days. Need for hospital stay and its duration was more in diabetics with UTI

Table 17: Association of age and ABO blood group of the patient with duration of Hospital stay as a categorical variable

Age range in years	Hospital stay (No admission)	Hospital stay (1-5 days	Hospital stay (>5 days)	ANOVA P value
13-25	3 (13.6%)	19(86.4%)	0(0%)	0.000
26-65	40(20.6%)	96(49.4%)	58(30%)	
66-85	11(14.1%)	34(43.6%)	33(42.3%)	
>85	0(0%)	0(0%)	6(100%)	
Total	54(18%)	149(49.7%)	97(32.3%)	
ABO Blood Group				
O	21(17%)	55(44.3%)	48(38.7%)	0.063
A	8(15.4%)	28(53.8%)	16(30.8%)	

B	21(21.7%)	56(57.7%)	20(20.6%)	
AB	4(14.8%)	10(37%)	13(48.2%)	
Total	54(18%)	149(49.7%)	97(32.3%)	

Table 18: Association of Sex of the patient with Duration of Hospital stay as a categorical variable

Sex	Hospital stay (0 days)	Hospital stay 1(1-5 days)	Hospital stay (>5 days)	Pearson Chi^2 P value
Male	23(18%)	66(51.6%)	39(30.4%)	0.818
Female	31(18%)	83(48.2%)	58(33.7%)	
Total	54(18%)	149(49.7%)	97(32.3%)	
Diabetic Status				
Non-Diabetic	37(19.6%)	112(59.3%)	40(21.1%)	0.000
Diabetic	17(15.3%)	37(33.3%)	57(51.4%)	
Total	54(18%)	149(49.7%)	97(32.3%)	

5.10 Association of virulence and antibiotic resistant factors of UPEC, diabetic status and renal dysfunction with duration of hospital stay

Duration of hospital stay was statistically influence by presence of NDM1 gene encoding Metallo β-lactamases.

Table 19: Association of virulence and antibiotic resistant gene of UPEC with duration of hospital stay as categorical variable

Gene	Duration of hospital stay in days			Wilcoxon rank sum (Mann-Whitney test) P value
	0	1-5	>5 days	
hlyA	19(22.6%)	39(46.4%)	26(32.3%)	0.428
cnf1	24(20.1%)	63(53%)	32(26.9%)	0.253
iss	17(25.8%)	31(47%)	18(27.3%)	0.163
fimH	12(19.3%)	33(53.2%)	17(27.4%)	0.650
papA	24(19%)	62(49.2%)	40(31.8%)	0.921
iutA	19(14.6%)	64(49.2%)	47(36.1%)	0.286
csgA	40(18.2%)	107(48.9%)	72(32.9%)	0.899
blaSHV	9(16.3%)	24(43.7%)	22(40%)	0.403
blaCTX-M	22(13.4%)	85(51.8%)	57(34.8%)	0.073
NDM1	0(0%)	7(38.9%)	11(61.1%)	0.012

Table 20: Association of diabetic status of patients and Renal dysfunction at presentation with need for hospital admission

Need for hospital admission	Diabetes mellitus		Pearson Chi2 P value	Renal dysfunction at presentation		Pearson Chi^2 P value
	Non-DM	DM		Absent	Present	
Absent	36(19%)	153(81%)	0.413	43(21.4%)	10(10.1%)	0.016
Present	17(15.3%)	94(84.6%)		158(78.6%)	89(89.9%)	
Total	53(17.7%)	247(82.3)		201	99	

Objective 5: RESULTS

To assess the ability of Neutrophil lymphocyte ratio and Urine microscopy score in independently predicting renal dysfunction at presentation and end of follow up, need for and duration of hospital admission

5.11 Association of Neutrophil lymphocyte ratio and Urine Microscopy score with renal dysfunction at presentation and at end of follow-up and duration of hospital stay

Table 21: Association of Urine Microscopy Score with Renal dysfunction at Presentation and at 14 days

UM score	Renal Dysfunction at presentation		P value	Renal Dysfunction at 14 days		Pearson Chi^2 P value
	Absent	Present	0.000	Absent	Present	0.000
1	201(91%)	20(9%)		217(98.2%)	4(1.8%)	
2	0(0%)	54(100%)		23(42.6%)	31(57.4%)	
3	0(0%)	25(100%)		0(0%)	25(100%)	
Total	201(67%)	99(33%)		240(80%)	60(20%)	

Note: Urine Microscopy scoring system (Table 1)

Table 22: Association of Neutrophil lymphocyte ratio with Renal dysfunction at Presentation and at 14 days

Renal Dysfunction at presentation	Neutrophil lymphocyte ratio		P value	Renal Dysfunction at 14 days	Neutrophil lymphocyte ratio		Two sample t test P value
	Mean	Std Dev	0.000		Mean	Std Dev	0.000
Absent (201)	3.486	0.456		Absent (240)	3.570	0.531	
Present (99)	4.407	0.778		Present (60)	4.668	0.735	

Total (300)				Total (300)			

Table 23: Association of Urine Microscopy score with duration of hospital stay

UM Score	Hospital stay in days			Pearson Chi2 P value
	0	1-5	>5 days	0.000
1	46(20.8%)	135(61.1%)	40(18.1%)	
2	8(14.8%)	13(24.1%)	33(61.1%)	
3	0(0%)	1(4%)	24((96%)	
Total	54(18%)	149(49.7%)	97(32.3%)	

Table 24: Association of Neutrophil lymphocyte ratio with duration of hospital stay as categorical variable

Hospital stay in days	Neutrophil lymphocyte ratio		One way Anova P value
	Mean	Standard Deviation	0.000
O	3.011	0.031	
1-5	3.50	0.335	
>5 days	4.66	0.468	

Discussion:

There are numerous studies which quote disease association with ABO blood group secretor status in saliva and other body fluids. Those individuals, whose body fluids are found to contain ABO blood group antigens are called Secretors and those who do not are called non-secretors. The presence of blood group antigens in body fluids and saliva is associated with margination and adhesion of various micro-organisms as well as inflammatory cells and cytokines. Thus, the categorization of patients as Secretor and non-secretor is used to study the predisposition towards several disease conditions, in particular infectious causes.

There are different methods to study the ABO secretor status in saliva or other body fluids. One of the common methods is adsorption-inhibition method by assessment of agglutination of red blood cells of respective blood group cells, when incubated in varying titers of saliva. Phenotypic method using adsorption-inhibition is a sensitive and specific method with ability for semi-quantitative assessment of the antibody titers in saliva. However, the constraint is that it is time consuming procedure and needs collection of saliva samples from all the patients Another approach is to study the various single nucleotide polymorphisms (SNP) associated with FUT2 gene for ABO blood group by polymerase chain reaction with gel electrophoresis of the products, from the deoxyribonucleic acid (DNA) isolated from peripheral blood mononuclear cells (PBMC). To cover both ABO and Lewis blood group antigens, combined study of polymorphisms in FUT2 and FUT3 gene is also undertaken. Since, the number of SNP's associated with FUT2 and FUT3 genes can be highly variable in different populations, several studies have been done to sequence the entire gene by both Sanger as well as by Next generation sequencing techniques.

ABO secretors are more commonly associated with H.pylori , HIV, Norovirus, Rotovirus infection, Irritable bowel disease.[16,17] On the other hand, non-secretors are more commonly associated with UTI, renal scarring, rheumatic fever and rheumatic heart disease.[18]

In present study with UTI patients, Non-secretors were of 61% whereas 18% in healthy controls which was similar to study from Himachal Pradesh where non secretors were 12% in healthy controls.[27]

In our study, UTI patients with blood group 'O' are more of Non-secretor. However, studies from other countries show O group more of secretor.[28] Thus, there is significant variation in the association of blood groups with the secretor status in body fluids. This difference may be due to racial variations, differences in methods used to study the secretor status in body fluids, phenotypic and/or genotypic methods, the number of single nucleotide polymorphisms studied to identify variations in ABO secretor status etc.

In our study in patients with UTI, 77.5% of diabetics were non-secretor, whereas a study from Nigeria show there is no association between secretor status and DM.[29]

There are several reports that non secretors are over expressed in UTI in women. Stapleton et al., stated that vaginal epithelial cells of non-secretor produce a unique sialosyl gal-globoside (SGG) and disialosyl gal-globoside (DSGG) which acts as receptor for adherence of E. coli.[30] E.coli, a motile bacillus remains the most common uropathogen. It migrates from rectum where it is a colonizer, to the urinary tract. Under favourable conditions, it migrate to upper urinary tract and causes pyelonephritis and renal dysfunction.

In our study, we found significant association of UTI due to UPEC with ABO non-secretor status in saliva. Ahmed T et al who found increased susceptibility to diarrhoea in children due to enterotoxigenic E.coli in non-secretors of Lewis blood group antigens.[31,32]

We studied various factors associated with bacterial virulence and antibiotic resistance such as serum resistance, biofilm production, UPEC with methicillin sensitive haemagglutination (MSHA) and UPEC with methicillin resistant haemagglutination (MRHA), and presence of hemolysin toxin by both phenotypic and biochemical methods as well as by studying the genes coding for these virulence and antibiotic resistance factors.

Serum resistance was reported in 80.7% of isolates in our study which shows its ability to resist host complement cascade. Similarly, a study from Kerala shows 63.5%.[33] Biofilm production was seen in 65.3% of UPEC in our study, while it was 45.6 % in a study from New Delhi.[34]

MSHA representing Type 1 fimbriae and MRHA representing P fimbriae were detected in 21% and 44.3% respectively which was similar in various studies. Hemolysin toxin was seen in 26% of the culture isolates.

Adhesion factors plays a pivotal role in invading the uroepithelium, csgA encoding Curli fimbriae was the most common gene detected whose role in enhancing biofilm formation was elaborated by various studies.[35,36] Its expression in E.coli in sepsis causes cytokine activation.[37] In our study, all the virulence factors studied by both phenotypic findings and the presence of corresponding genes, were significantly influenced by non-secretor status, except serum resistance. Diabetic status doesn't affect the expression of any of the virulence factors in present study.

Antibiotic resistance study by Kirby bauer shows higher level resistance to ampicillin, cotrimoxazole, norfloxacin and ceftriaxone with lower level of resistance to amikacin, nitrofurontion and meropenem. Fosfomycin was a older drug which was approved by US Food and drug Administration (FDA) for single dose therapy in Uncomplicated UTI. Our study shows 100% sensitivity to Fosfomycin and other studies shows as low as 1% resistance to Fosfomycin in E.coli.[38,39] It also proves its efficacy even in ESBL producing organisms

Derington CG et al 2020 has proved the efficacy of Multi dose Fosfomycin in complicated UTI.[40] The presence of genes bla CTX-M and NDM1 were significantly associated with blood group non-secretor status and resistance to β-lactamaese group of drugs.

In our study in patients with UTI due to UPEC, we assessed the presence of renal dysfunction at presentation and at the end of 14 days follow up for all the patients. We found that, older the patient, the risk of renal dysfunction was significantly higher, both at presentation and at the end of 14 days follow up and this finding was found to be statistically significant. In our study, paediatric age group was not studied. In adult population, the association of UPEC with older age group was similar to the findings in Terlizzi et al, 2017.[41]

In our study, neutrophil-lymphocyte ratio in complete hemogram, at the time of diagnosis of culture proven UTI due to UPEC in patients with significant bacteriuria was found to be significantly associated with the need for hospital admission, duration of hospital admission, and association with renal dysfunction at presentation as well as with persistent renal dysfunction at the end of 14 days of follow up. In a study from Sharjah, United Arab Emirates, Saheb Sharif-Askari et al, found higher neutrophil lymphocyte ratio to be associated with ESBL producing E.coli, particularly in patients with more severe renal disease and higher mortality rate. They had also found NLR to be significant in predicting the disease outcome in patients with ESBL E.coli among diabetics. However, they did not find any statistical association with K.pneumoniae UTI.[25] However, in our study, we correlated the higher NLR scores at diagnosis with evidence of renal dysfunction and requirement of hospital admission at the time of diagnosis as well as on follow up and we found it to be a useful predictor of the severity of infection and its ability to cause visceral damage to the kidney.

Perazella had proposed study of tubular epithelial cells and granular casts in urine microscopy with a semi-quantitative scoring system as a useful and sensitive tool to screen for presence of renal injury and renal dysfunction in various renal diseases.[12] We had studied the utility of

Perazella Urine microscopy scoring system in patients with UTI due to UPEC and found the score to be a very useful tool in significantly predicting the need for hospital admission, duration of hospital admission, renal dysfunction at presentation as well as with persistence renal dysfunction at the end of 14 days follow up. In one of the studies from our own group, we found that NLR at presentation was an early predictor of acute kidney injury due to sepsis.[14] Similar predictive ability of Urine microscopy scoring system was found in sepsis induced AKI in several other studies.[26]

Conclusion:

We studied 300 patients with culture proven UTI due to Uropathogenic E.coli and assessed the ABO and Rh blood group secretor status in saliva by adsorption inhibition method. Females constituted 57% and 82% of our patients needed hospitalization for various indications. Diabetics constituted 37% of the study population. Renal dysfunction was present in 33% of the study population at presentation and it was persistent in about 20% of the patients at the end of 14 days follow up after management of UTI. Blood group O was significantly associated with blood group non-secretors while such association was not significant with other blood groups. Older age, diabetics and patients with blood group O were found to have statistically significant association with longer duration of hospital admission, while older age was associated with presence of renal dysfunction both at presentation and at 14 days follow, female sex was associated significantly with presence of renal dysfunction at presentation alone. Non-secretors who had absence of ABO blood group antigens in saliva were significantly associated with diabetes mellitus and were also at a higher risk for UTI and renal dysfunction when compared with blood group secretors. In culture isolates from study subjects, serum resistance was reported in around 81% while biofilm production by Crystal violet method was observed in 65%. MSHA and MRHA were observed in 21% and 44% of the patients and hemolysin toxin was identified in 26%. All the virulence factors studied by both biochemical findings and the corresponding genes hlyA, cnf1, iss, fimH, papA, iutA and csgA were significantly associated with ABO blood group non-secretors while there was no significant association with diabetes mellitus. Antibiotic resistance for ampicillin, ceftriaxone and ESBL producing antibiotics are significantly associated with non-secretors while amikacin, co-trimoxazole, nitrofurantoin, norfloxacin, and meropenem were not found to have statistically significant difference in antibiotic resistance between blood group secretors and non-secretors. We found an interesting observation that Fosfomycin resistance was not observed in any of our culture

isolates, emphasizing the antibiotic sensitivity for older antibiotics. Genes blaSHV, blaCTX-M and NDM1 studied for antibiotic resistance, showed statistically significant association of non-secretors with blaCTX-M and secretors with NDM1 gene. Type 2 diabetes mellitus was found to be significantly associated with renal dysfunction at presentation and at 14 days follow up while non-secretors were found to be statistically significant with renal dysfunction at presentation. Presence of bacterial virulence factors or antibiotic resistance genes were not found to have statistically significant association with renal dysfunction at presentation or at 14 days follow up. Among all the virulence and resistance genes, only NDM1 was significantly associated with longer duration of hospital stay due to UPEC infection. Patients with older age and presence of type 2 diabetes were also significantly associated with longer duration of hospital stay. At the same time, neither age, sex, nor diabetes mellitus were associated with need for hospital admission while presence of renal dysfunction at presentation was significantly associated with hospitalization. Quantitative urine microscopy by Perazella scoring system to assess the presence of renal tubular epithelial cells and granular casts and neutrophil-lymphocyte ratio at diagnosis were found to be significantly useful predictor for presence of renal dysfunction both at presentation as well as at 14 days follow up. Both urine microscopy score and NLR at time of diagnosis were found to significantly predict the need for hospital admission and duration of hospital stay in patients with UTI due to UPEC.

Limitations:

- Pediatric population and pregnant women with UTI were not studied
- In view of the referral nature of the Institution, majority of the patients had more serious disease requiring hospitalization and this constituted referral bias and influenced the patients included in the study
- Blood group secretor status in saliva was assessed only for ABOH groups and not for P, Lewis or other blood group antigens.
- Blood group secretor status was not assessed for polymorphisms in FUT2 and FUT3 genes either by polymerase chain reaction or by Next generation sequencing
- Follow up of the patients was limited to 14 days as we lost follow up beyond that period for significant proportion studied.
- Detailed analysis of the patient's underlying renal disease was not a part of the study and could be a confounder, at least in a small subset of the patients.

Summary:

- Study population of 300 adult subjects with UTI due to Uropathogenic E.coli.
- Females were most commonly affected and diabetics constituted 37% of study participants
- ABO blood group non-secretors and patients with blood group O were more commonly affected by UTI due to UPEC.
- 82% of our study participants needed varying duration of hospital admission for management.
- Renal dysfunction was present in 33% of study participants at presentation and persisted in 20% at the end of 14 days follow up.
- Female sex commonly had renal dysfunction at presentation while old age was significantly associated with renal dysfunction both at presentation as well as at the end of 14 days follow up.
- Non secretors statistically associated with presence of type 2 diabetes and both were strongly associated with presence of renal dysfunction.
- Patients with older age, type 2 diabetes and blood group O were significantly associated with longer duration of hospital admission.
- In culture isolates, serum resistance was seen in 81%, biofilm production by 65%, MSHA and MRHA in 21% and 44% respectively and hemolysin toxin identified in 26%.
- All bacterial virulence factors and corresponding genes hlyA, cnf1, iss, fimH, papA, iutA and csgA were significantly associated with ABO blood group non-secretors. Bacterial virulence factors had no significant association with diabetes.
- Antibiotic resistance for ampicillin, ceftriaxone and ESBL producing antibiotics are significantly associated with non-secretors

- Antibiotic fosfomycin was found to be 100% sensitive in our culture isolates.
- Antibiotic resistance gene blaCTX-M significantly associated with non-secretor and NDM1 with secretors.
- Bacterial virulence factors and antibiotic resistance were not found to be significantly associated with renal dysfunction at presentation and at follow up.
- Only NDM1 gene was significantly associated with longer duration of hospital admission in UTI patients
- Presence of renal dysfunction at presentation was significantly associated with need for hospitalization.
- Neutrophil-lymphocyte ratio at the time of diagnosis and quantitative urine microscopy scoring by Perazella system are found to significantly predict renal dysfunction at presentation and at follow up as well as the need for hospitalization and duration of hospitalization for management of UTI.

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Conferences Attended and Presentations:

S.No	Name of the CME/Conference	Date and Venue	Presentations
1	Hospital Acquired Infection – Diagnosis and Surveillance	17th November 2017, Indira Gandhi Medical College and Research Institute, Puducherry	
2	Microcon 2018 – 42nd Annual Conference of Indian Association of Medical Microbiologists	28.11.2018 to 2.12.2018, NIMHANS, Bengaluru	Oral Presentation – Does Secretor status of ABO blood group in saliva influence the risk of hypertension and urinary tract infection in diabetic patients? On 1.12.2018
3	Workshop on Emerging Zoonotic Diseases and Inter-sectoral coordination for Medical and Veterinary Professionals	26.03.2019, Puducherry State Health Mission, Puducherry	
4	Antimicrobial stewardship and Infection control strategies 2019 (ASICS 2019)	19.06.2019, Mahatma Gandhi Medical College and Research Institute, Sri Balaji Vidyapeeth, Puducherry	Podium Oral Presentation – Distribution of extended spectrum Beta lactam and Carbapenam resistance genes and genetic diversity among Uropathogenic *E.coli* isolates in Puducherry, India. On 19.06.2019
5	Thesis Writing Workshop	16th and 17th October 2020 at Sri Balaji Vidyapeeth, Puducherry	
6	Training on use of Integrated Health Information Platform in Integrated Disease Surveillance Program	12th and 13th November 2019, NHM Training Hall, DHFWS, Puducherry	
7	National Workshop on Guidelines for applying Extramural Research Grants: a faculty Development Program	19th and 20th November 2019 at Sri Balaji Vidyapeeth, Puducherry	
8	CIDSCON 2020 – 10th Annual conference of Clinical Infectious Diseases Society	21-29th November 2020; Virtual meet	